

pending claims. The pending claims are shown above. No new matter has been added.

The objections to claims 51-86 stated in paragraph 3 of Paper No. 14 are moot in view of the above. The claims have been amended above with the Examiner's comments in mind.

The Section 112, first paragraph, rejection of claims 69-73, 81 and 82, stated in paragraph 5 of Paper No. 14 is moot in view of the above. The pending claims are supported by an enabling disclosure and the Examiner is requested to consider the following in this regard.

The present invention relates to mononuclear phagocytes modified to comprise at least one hypoxia and/or ischemic and/or stress regulatable element operably linked to at least one nucleotide sequence of interest (NOI) and their use in methods of targeting a mononuclear phagocyte to hypoxic and/or ischemic and/or stress sites, methods of treating a condition associated with a hypoxic and/or ischemic and/or stress state as well as a method for selectively destroying a mononuclear phagocyte and a delivery system comprising said mononuclear phagocyte.

The Examiner has rejected now-canceled claims 69-73, 81 and 82 as containing subject matter not described in the invention in such a way as to enable the skilled person to use the invention. The pending claims relate to a method for selectively destroying a mononuclear phagocyte and to a method for targeting a mononuclear phagocyte to hypoxic and/or ischemic and/or stress sites. The claimed invention further provides a method for treating a condition associated with a

and/or stress state as well as to an *ex vivo* method for treating a condition associated with a hypoxic and/or ischemic and/or stress state. The presently claimed invention further provides a delivery system for targeting a mononuclear phagocyte of the invention to a target hypoxic and/or ischemic and/or stress site.

The Applicants submit that the present invention is sufficiently exemplified to enable a person of ordinary skill in the art to make and use the claimed invention. The specification provides extensive guidance on the preparation of the mononuclear phagocytes of the invention and their uses in methods of the claimed invention.

The specification teaches that a binding agent, for example a viral vector, may be used to introduce a hypoxia and/or ischemic and/or stress regulatable agent comprising a therapeutic gene into a mononuclear phagocyte. Furthermore, it is well known in the art that genes may be transferred into non-dividing cells such as monocytes/macrophages using a replication defective adenoviral vector.

Methods of *in vivo* and *ex vivo* gene delivery/gene transfer are clearly described in the application. For example, methods for *in vivo* gene transfer are described on page 38 with methods for *ex vivo* gene transfer described, for example, on pages 37-38. The specification describes methods of gene delivery such as the preparation of a retroviral vector and its transfer to U937 monocytic cell lines (see page 21, Example 1) and gene transfer to primary human macrophages using an adenoviral vector (Example 2). As exemplified in the specification, efficient transfer of genes into human macrophages has been achieved using a replication defective adenoviral vector with expression of the gene in 40-80% of the cells over a period of time.

weeks after gene transfer (see page 36, lines 17-20). In addition, transfected monocytes/macrophages have been injected directly into disease tissue of a donor (see page 38, lines 3-5).

Example 5 demonstrates the effect of clamp induced hypoxia on macrophage infiltration into tumour xenografts. The clamping of the tumours induces hypoxia in the tumours which led to increased infiltration of macrophages (see page 33, lines 23-24). This result demonstrated a correlation between the degree of hypoxia and the number of infiltrating macrophages. This result strengthens the applicants' findings that mononuclear phagocytes may be used to deliver drugs to hypoxic/ischemic sites where mononuclear phagocytes are typically present (see page 5, lines 22-25).

Thus, the applicants submit that detailed guidance for the preparation of mononuclear phagocytes of the present invention and their use in methods of the claimed invention have been provided in the Examples described on pages 36-39, for example, and the disclosure is enabled by virtue of these examples as well as the generally advance knowledge in the art at the time of the present invention..

Beyond the teachings of the present disclosure, the applicants now provide additional data in the attached Declaration of Stuart Naylor, Ph.D. It is submitted that the data clearly demonstrates:

- (i) transduction of macrophages with adenoviral vectors comprising either a hypoxic regulatable (HRE) reporter gene (such as Lac Z) or CMV regulated reporter genes (such as GFP);

- (ii) subcutaneous injection in the periphery of a tumour/intratumoural injection of macrophages comprising either HRE regulated reporter gene (such as Lac Z) or CMV regulated reporter genes (such as GFP) into a murine xenograft model and intraperitoneal ovarian cancer model; and
- (iii) hypoxic regulated expression of the reporter gene (such as LacZ) in the tumour site.
- (iv) an adenoviral transduced macrophage can deliver a nucleotide sequence of interest(NOI) to a target site; and
- (v) the NOI delivered by adenoviral transduced macrophage can be selectively expressed at a target site such as a hypoxic site when the expression of the NOI is regulated by a hypoxic response element (HRE).

Accordingly, it is submitted that the presently claimed invention is enabled by the present application as well as the generally advanced level of skill in the art at the time of the present invention. All claims are submitted to be supported by an enabling disclosure.

The applicants also submit that the "sustained expression" concerns raised by the Examiner in relation to the Verma citation are not relevant to the presently claimed invention because there is a continual turnover of monocytes/macrophages in the blood stream and at diseased tissue sites. Thus, the continual infiltration of monocytes/macrophages from the blood stream provides a sustained expression of the gene of interest *via* a short term expression from each transduced/transfected cell.

With respect to the Examiner's specific concerns as to the relevance of studies using nude mice, the Examiner is urged to consider the following:

Firstly, in order to be of any clinical utility, a scaleable process needs to be established, that can be translated to human application. In order to determine the inherent potency of this technology rather than indirect immune effects the preferable model is to use human macrophages administered to human tumours thus an immunocompromised background is required. For the practibility of numbers and experimental significance the nude mouse model is the *in vivo* model of choice. In contrast, the use of *in vivo* modeling in a syngeneic background will introduce parameters that will cloud the inherent potency of the system, i.e., immune activation.

Secondly, gene therapy techniques may be performed in conjunction with at least some degree of immunosuppressive treatment to maximize the success of the therapy. Therefore, the use of a model in which the immune system is compromised such as that in the nude mouse is a valid model.

Thirdly, in a recently publicized news article describing gene therapy success, a child has been successfully cured of severe combined immunodeficiency (SCID), a condition in which the child is born with no immune system of their own and becomes highly vulnerable to infection as they lose maternal immunity. (see http://news.bbc.co.uk/hi/english/health/newsid_1906000/1906999.stm – a copy of the article is attached for the Examiner's convenience and consideration).

For these reasons, the applicants believe that the results of the study described in the application are relevant to the Examiner's concerns.

The claims are submitted to be supported by an enabling disclosure.

The Section 112, second paragraph, rejections of claims 51-85 stated in paragraph 7 of Paper No. 14 and of claims 68, 69 and 70, stated at paragraph 8 of Paper No. 14, are moot in view of the above. The pending claims are submitted to be definite. Consideration of the following in this regard is also requested however.

The Examiner has objected to the use of the phrase "hypoxia and/or ischemic and/or stress regulatable agent" throughout the claims as being indefinite. The claims have been amended to refer at least one regulatable element selected from the group comprising a hypoxia regulatable element, a ischemic regulatable element and a stress regulatable element, to advance prosecution, without prejudice.

Clear guidance as to what constitutes hypoxic conditions, ischemic conditions and stress conditions are given in the specification (see for example, page 2 paragraphs 1 and 2, page 6 paragraph 1, page age 9 paragraph 3). It is well known that there is some degree of overlap between conditions in which hypoxia, ischemia and stress occur and in which the present invention may be used. For example, as indicated on page 2 line 3, hypoxia is often accompanied by hypoglycaemia, which is also a feature of ischemia and stress (see page 2 lines 12 to 16). It is submitted that the ordinarily skilled person appreciates the interrelationship of hypoxia, ischemia and stress and will accordingly understand that the invention is not restricted to the use of a single regulatable element or indeed a particular combination.

The Examiner's objection to now-canceled claims 60 and 77 has been addressed by not repeating the recitation of "preferably" clauses and making such clauses the bases of new dependent claims. No new matter has been added.

It is submitted that, contrary to the opinion of the Examiner, the phrase "bioreductively activated pro-drug" as used in the claims is defined in the present application. Page 9 lines 3 to 6 defines bioreductively activated prodrugs which may be used in the invention as prodrugs which are activated at very low levels of oxygen as well as with contact with enzymes such as reductases. and, in addition, gives examples of such prodrugs.

It is submitted that the meaning of the term "activating or control product" in the claims is well known in the art and is self-explanatory and, moreover is clear from its context in the claims and description of the present application. Page 11, line 19 to page 20 line 18 describes the use of inducible or repressible promoter elements to control the expression of a therapeutic gene. For example, as described on page 12 lines 2 to 18, the use of a DNA sequence encoding a tetracycline repressor protein to repress transcription of a therapeutic gene is described. Page 14, line 24 to page 15, line 11, describes the use of further control and activating elements for regulating expression of a gene. In particular, reference is made to the use of genes encoding transcription factors, such as HIF1-alpha, to enhance the response to hypoxia, stress or low glucose. Furthermore, should the skilled person need any further guidance as to the meaning of these terms, the claims provide such guidance as they are directed to

embodiments in claim 10.

tetracycline repressor protein respectively. Thus, it is submitted that the term is clear to the person of ordinary skill in the art, both from common knowledge in the art and from the description and claims of the specification.

The Examiner's comments regarding now-cancelled claims 68, 69 and 70 as allegedly having been incomplete based on the use of "provided" or "providing" has been addressed in the pending claims as the pending claims recite that the mononuclear phagocyte further comprises an NOI encoding a protein.

However, with respect the Examiner's criticisms of now-canceled claims 69 and 70, the applicants submit that the Examiner's objection was unreasonable. In these claims the methods required as a first step that a mononuclear phagocyte be provided in order that the mononuclear phagocyte can be exposed to hypoxic and/or ischemic and/or stress conditions or be allowed to migrate. The term "provided" is thus being used according to its normal usage, which is commonplace in patent practice and, as such, would be readily understood by a person of ordinary skill in the art. There are thus no omitted elements.

The claims are therefore submitted to be definite.

The Section 101 rejection of claims 51-53, 56, 60-65, 68 and 70, is moot in view of the above. The pending claims are submitted to define patentable subject matter.

Specifically, as described above, the claims provide a modified mononuclear phagocyte, wherein said modification comprises at least one hypoxia and/or ischemic and/or stress regulatable element operably linked to at least one nucleotide sequence of

The Section 102 rejection of claims 51-54 and 68 over Ferkol *et al* ((1996) (PNAS 93 101-105) is moot in view of the above. The pending claims are submitted to be patentable over the cited art and the Examiner is requested to consider the following in this regard.

Ferkol *et al* describes a non-viral method for introducing genes into a macrophage cell using receptor-mediated gene transfer. Specifically, Ferkol *et al* (1996) describes *in vitro* and *in vivo* gene transfer into macrophages using a non-viral system by specifically targeting the mannose receptor in macrophages.

Ferkol *et al* (1996) does not disclose or suggest the preparation of mononuclear phagocytes modified to comprise a hypoxia and/or ischemic and/or stress regulatable element operably linked to a nucleotide sequence of interest (NOI) or the use of a mononuclear phagocyte comprising a hypoxia and/or ischemic and/or stress regulatable element operably linked to nucleotide sequence of interest (NOI) to regulate the expression of an NOI at a target site where hypoxia and/or ischemic and/or stress conditions may be present. Indeed, as described on page 101, col 2, para 4 of Ferkol *et al* (1996), the expression plasmids used contained a SV40 promoter and enhancer ligated to a luciferase gene and a CMV promoter ligated to a LacZ gene. Neither of these promoters or enhancers constitute a hypoxia and/or ischemic and/or stress regulatable element as required by the pending claims.

Therefore it is submitted that the claims are not anticipated by Ferkol *et al*.

The Section 103 rejection of claims 51-68, 74-80 and 93-86 over Ferkol (US

view of the above. The claims are submitted to be patentable over the combination of art and consideration of the following in this regard is requested.

Leek *et al* (1996) was published on October 15, 1996. The priority date of the present application is October 9, 1996 and thus predates Leek *et al*. It is submitted that the subject matter of the invention is entitled to the October 9, 1996 priority date and thus Leek *et al* (1996) is not citeable prior art. A copy of the priority document has been received by the USPTO. See, Notification dated May 28, 1999.

Moreover, as described below, even if Leek *et al* were available as citeable prior art against the present application, which, as detailed above, it is not, the combination of the disclosures of Leek *et al* with that of Ratcliffe *et al* and Ferkol *et al* is based on impermissible hindsight and would, in any case, not have led the ordinarily skilled person to the presently claimed invention.

Specifically, the applicants submit that the presently claimed invention is neither disclosed nor suggested in Ratcliffe *et al*, Ferkol *et al* either individually or in combination thereof.

In this respect Ratcliffe *et al* teaches nucleic acid constructs comprising at least one gene encoding a species having activity against disease, operatively linked to a hypoxically inducible expression control sequence. Ratcliffe *et al* provide teachings relating to cancer cell line, fibroblast cell lines and tumour cells transfected with a construct which were then implanted under the skin of a mouse. Thus, Ratcliffe *et al* teaches that tumour cells and/or cell lines can be modified under *in vitro* conditions. However, Ratcliffe *et al* is silent with regard to

tumour cells, such as macrophage cells, with a hypoxic construct. Moreover, Ratcliffe *et al* is silent with respect to modifying monocyte/macrophage cells with a viral vector comprising a hypoxic construct, which is capable of transducing such cells, or any advantage/beneficial effects that might be associated with such modifications. Without such teaching in Ratcliffe *et al*, for example, the ordinarily skilled person would not have been motivated to modify the teachings in Ratcliffe *et al* to arrive at the presently claimed invention.

The applicants submit that Ferkol *et al* (US 5,972,900) addresses a different problem to the presently claimed invention and to that of Ratcliffe as it relates to an approach to the treatment of storage diseases that affect the reticuloendothelial system, for example, Gaucher disease using the macrophage as "a primary target for genetic correction". (col 31 line 48).

Gaucher Disease is a rare autosomal recessive disorder characterized by defective function of the catabolic enzyme beta-glucocerebrocidase leading to an accumulation of glucocerebroside in cells of the monocyte-macrophage system. The teachings of Ferkol *et al* with respect to genetic manipulation of macrophages are therefore concerned with corrective transformation of the macrophage in order to treat this inborn error of metabolism of those cells. There is no suggestion that such a method be adapted to target gene expression from macrophages to a particular site such as an hypoxic site, such as a tumour.

In addition, the teachings in Ferkol *et al* (US 5,972,900) are directed to a non-viral method for introducing genes into

transfer. Ferkol *et al* (US 5,972,900) do not disclose or suggest either the modification/manipulation of monocytes/macrophages with a viral vector comprising a hypoxic construct, which is capable of transducing such cells, or any advantage/beneficial effects that might be associated with such modifications.

In summary, Ferkol *et al* (US 5,972,900) provides no indication that mononuclear phagocytes may be used to adapted to target gene expression from macrophages to a particular site such as an hypoxic site. It is simply concerned with genetic modification of macrophages to correct a genetic error in those cells i.e. using the macrophages as a primary target for therapy. Ratcliffe *et al* as described below teaches the genetic modification of tumour cells, wherein the tumour cells are primary targets for therapy. Again, the applicants submit that Ratcliffe provided no motivation to the ordinarily skilled person to adapt the teachings therein to use macrophages to express genes at a target site such as an hypoxic site. Thus, the ordinarily skilled person would not have considered combining the teachings of these two documents.

In fact, the teachings in Ferkol *et al* would have discouraged the ordinarily skilled person from combining the teachings in Ferkol *et al* with the teachings in Ratcliffe *et al* because Ferkol *et al* state in col 31, lines 49-55 that: "*practical questions regarding the efficiency and specificity of gene delivery using this system (ie gene transfer via the mannose receptor) need to be addressed*". Thus, any attempt to combine the teachings in Ferkol *et al* with Ratcliffe *et al* would have run contrary to the teachings in Ratcliffe *et al* because Ferkol *et al* report that expression of a reporter gene can be detected in the liver and spleen of mice when a complex of

and mannosylated polylysine is infused into mice. That is, the complex migrates to sites where there is an abundance of tissue macrophages because the mannose receptor fused to the reporter complex mediates gene transfer into macrophages. Thus, even if the method of gene delivery in Ferkol *et al* were successful, it would not have persuaded the ordinarily skilled person to prepare the composition of the presently claimed invention, for delivery of a gene of interest, such as a therapeutic gene, to a hypoxic site which may not be a site such as liver or spleen which has an abundance of tissue macrophages.

As described above applicants submit that Leek *et al* is not citeable prior art against the present application. However, even if Leek *et al* was available as citeable prior art against the present application, which it is not, the combination of the disclosures of Leek *et al* with that of Ratcliffe *et al* and Ferkol *et al* is based on impermissible hindsight and, moreover, even if they were combined, would not have led the ordinarily skilled person to the presently claims invention.

Specifically, Leek *et al* relates to the use of the macrophage as a target immuno-inhibition therapy in breast cancer (see last line of the abstract on page 4625). In this regard, Leek *et al* suggest that tumour associated macrophages (TAMs) could be an effective target using agents such as IL-10 or Linomide which block macrophage infiltration. Thus, the Leek *et al* citation provides teachings on how to control i.e. prevent) macrophage migration, rather than enhance macrophage migration, to a target site, such as a tumour site, in order to deliver a gene to selectively destroy either a tumour cell and/or a monocytes/macrophages at the target site.

It is clear from Leek *et al* that there would have not been any motivation for the ordinarily skilled person to look in this citation to adapt the teachings of either Ratcliffe *et al* or Ferkol *et al* because Ratcliffe *et al* relates to the selective expression of a gene from a tumour cell and Ferkol et al merely relates to the delivery of a gene construct to correct an error of metabolism in macrophage cells. In contradistinction, the presently claimed invention relates to the selective expression of a hypoxic and/or stress and/or ischemic regulatable gene from a macrophage which is either present at the target site or targeted to the target tumour site from a remote site.

In summary, any attempt to combine the teachings in Leek *et al* with the teachings in Ratcliffe *et al* and/or Ferkol *et al* would not have led the ordinarily skilled person to the presently claimed invention. Moreover, as described above, Leek *et al* is not citeable prior art against the present application, and thus the combination of its teachings with that of Ratcliffe *et al* and/or Ferkol *et al* is improper. Thus, the claims are submitted to be patentable over the Examiner's combination of art.

The claims are submitted to be in condition for allowance and a Notice to that effect is requested.

LEWIS et al
Serial No. 09/284,009

Respectfully submitted,

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